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3D microfluidic origami electrochemiluminescence immunodevice for sensitive point-of-care testing of carcinoma antigen 125

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ABSTRACT

In this paper, a 3D microfluidic origami device combined with electrochemiluminescence (ECL) immunosensor was introduced for sensitive point-of-care testing of carcinoma antigen 125 in clinical serum samples for the first time. This microfluidic origami device was fabricated by directly screen-printing carbon working, counter electrodes and Ag/AgCl reference electrode including their conductive pads on wax-patterned pure cellulose paper, and was activated by folding to form a 3D electrochemical cell. To construct a sensitivity-enhanced sandwich-type ECL immunosensor, gold nanoparticles (AuNPs) was synthesized to modify the working electrode to provide a good pathway of electron transfer and enhance the immobilized amount of capture antibody ($McAb_1$), and luminol functionalized gold nanoparticles (Lu-AuNPs) was synthesized to label signal antibody ($McAb_2$). With the aid of a facile device-holder, cyclic voltammetry was applied on microfluidic origami ECL immunodevice to trigger ECL. Under optimal conditions, this microfluidic origami ECL immunodevice provided a good linear response range from 0.01 to 100 U mL^{-1} with a detection limit of 0.0074 U mL^{-1}. The proposed ECL immunodevice presented high sensitivity and stability, and could be applied in point-of-care testing of other tumor markers for remote regions and developing countries.

1. Introduction

In the tumorous process, increased levels of tumor markers, including proteins, enzymes and peptide hormones, in human serum are significantly associated in patients with certain tumor or carcinoma. Thus, rapid, quantitative and sensitive detection of tumor markers in human serum is essential for early, prompt and effective diagnosis and treatment [1]. Immunoassay is a common bio-analytical method that measures tumor markers using the reaction of an antibody to its antigen [2]. At present, the clinical analyses such as fluoroimmunoassay (FIA) [3], enzyme-linked immunosorbent assay (ELISA) [4,5], mass spectrometric immunoassay (MS) [6] and chemiluminescence immunoassay (CLIA) [7] carried out in developed economies are often not applicable in developing countries, because these analytical systems are too expensive, large, complicated, and dependent on specific infrastructures. Therefore, it is of considerable interest to the further research for sensitive, accurate, rapid and simple alternative methodology for determination of tumor markers.

Microfluidic devices [8] have emerged as privileged devices in integrated analytical systems [9], and the small reaction volume and microchannel dimensions offer the advantage of strong analysis time reduction but minute quantities of samples and reagents (typically a few hundred nanoliters) [10–13]. Recently, microfluidic paper-based analytical devices (μPADs) have gained more and more attention and great interest [14–17], which are capable of quantifying the concentrations of various analytes in aqueous solutions, including biological fluids such as urine, serum and blood. Importantly, μPADs can be fabricated in bulk using less expensive means, including photolithography [18], polydimethylsiloxane (PDMS) plotting [19], inkjet etching [20], plasma etching [21], cutting [22], and printing [23]. Among these fabrication methods, wax-printing using a commercially available wax printer is among the cheapest and most easily implemented means of mass production available, and has recently been shown to be effective in the production of μPADs at minute cost.

The primary detection method for the qualitative analysis of analytes on μPADs is colorimetric method [24]. However, the conventional qualitative "yes/no" answers [15], low sensitivity and high limit of detection of colorimetric method have

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prevented the μPADs from reaching their full potential and competing with the traditional analytical instrumentations [25,26]. Electrochemiluminescence (ECL), which combines the advantages of chemiluminescence and electrochemistry, continues to impact diverse areas ranging from chemical analysis to the molecular-level understanding of biological processes [27]. Recently, the establishment of ECL on μPADs [28] based on the integration of μPADs and screen-printed electrodes have substantially increased the scope of options for detections on μPADs, and shown excellent prospects for analyte detection.

To further perform high-performance, sensitive-enhanced sandwich-type ECL immunoassays, attention has been focused on innovative approaches that couple immobilization platforms and amplification processes [29]. By incorporation with nanoparticles (NPs), the formations of NPs/biomolecule conjugation and NPs amplifying label platform enhance the signal, and provide the basis for ultrasensitive immunoassays [30–32]. For immobilizing biomolecules, gold nanoparticles (AuNPs) have been recognized as a versatile and efficient platform for the conjugation of biomolecules for their good electroconductibility, large specific surface area and versatility in surface modification [33]. For signal amplification, luminol functionalized gold nanoparticles (Lu-AuNPs) have been used as ideal material for labeling, which not only retain the high-efficiency ECL property of luminol, but also own the good stability and simple coupling procedure to antibody or antigen of AuNPs [34]. Herein, we use AuNPs and Lu-AuNPs to obtain enhanced sandwich ECL immunosensor.

In this paper, we designed a sandwich-type ECL immunosensor for determination of carcinoma antigen 125 (CA125) by combining a 3D microfluidic origami device. Using wax-patterned paper fluidic substrates and screen-printing electrodes, we constructed low cost, disposal, portable microfluidic origami device. AuNPs were used as immobilization platform to improve electron transfer and enhance the immobilized amount of capture antibody ($McAb_1$). Lu-AuNPs were synthesized as signal amplification section to label signal antibody ($McAb_2$). After a folding procedure, the prepared ECL immunosensor was put into a home-made device-holder and ready for CA125 detection. Ultimately, cyclic voltammetry was applied for ECL detection. The aim of this study is to explore simple, sensitive, low-cost, disposable and portable point-of-care testing devices.

2. Materials and methods

2.1. Reagents

All other reagents were of analytical grade and used as received, and all solutions were prepared using Millipore (model Milli-Q) purified water. Carcinoma antigen 125 (CA125), capture anti-CA125 antibody ($McAb_1$) and signal anti-CA125 antibody ($McAb_2$) were purchased from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). L-Cysteine (L-Cys) and bovine serum albumin (BSA, 96–99%) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). $HAuCl_4$, luminol, nitric acid, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were obtained from Alfa Aesar China Ltd. (Tianjin, China). Whatman chromatography paper #1 (20.0 cm × 20.0 cm) (pure cellulose paper) was obtained from GE Healthcare Worldwide (Pudong Shanghai, China) and used with further adjustment of size. Carbon ink (ED423ss) and silver/silver chloride (Ag/AgCl) ink (CNC-01) were purchased from Acheson. Blocking buffer for blocking the residual reactive sites on the antibody immobilized paper was pH 7.4 phosphate buffer solution (PBS) containing 0.5% BSA and 0.5% casein. The clinical serum samples were from Shandong Tumor Hospital.

2.2. Apparatus

The ECL measurements were carried out on an MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remex Analytical Instrument Ltd., Co.) equipped with a photomultipier tube (PMT, detection range from 300 to 650 nm) biased at 800 V. Transmission electron microscopy (TEM) images were obtained from a Hitachi H-800 microscope. Scanning electron microscopy (SEM) images and energy-dispersed spectrum (EDS) experiment were recorded on a JEOL JSM-5510 scanning electron microscope. A commercially available wax printer (FUJIXEROX Phaser 8560DN, Japan) was used for wax-printing chromatography paper.

2.3. Design and fabrication of this 3D microfluidic origami device

This 3D microfluidic origami device was fabricated on pure cellulose paper, and the fabrication process could be finished within 10 min. The fabrication procedure (Scheme 1) was described as follows: firstly, the configuration for wax-patterning on rectangular paper sheet (20.0 cm × 20.0 cm) was designed using Adobe illustrator CS4, and a wax printer was used for wax-patterning in bulk (Procedure (1) in Scheme 1). The wax-patterned paper sheet was baked in an oven at 130 °C for 150 s to let wax melt and penetrate through the paper. On each wax-patterned paper (60.0 mm × 30.0 mm), there were two circular paper working zones (named circle-A: 6.0 mm in diameter and circle-B: 8.0 mm in diameter) for screen-printing electrodes (Procedure (2) in Scheme 1). In circle-A, carbon ink was used for screen-printing working electrode (4.0 mm in diameter); in circle-B, carbon ink and Ag/AgCl ink were, respectively, used for screen-printing half-ring like counter electrode and reference electrode. Then, the conductive wires and contact pads were screen-printed in the defined area. After that, the prepared paper sheet was cut to rectangular paper (60.0 mm × 30.0 mm) (Procedure (3) in Scheme 1). Finally, by folding the rectangular paper in half, a 3D microfluidic origami device with an electrochemical cell of ~30 μL was obtained.

2.4. Preparation of AuNPs modified platform

AuNPs were synthesized by sodium citrate reduction of $HAuCl_4$ in water [35]. Briefly, 0.5 mL of 2% sodium citrate was rapidly added to 50 mL of boiling 0.01% $HAuCl_4$ solution under vigorous stirring, and then the solution changed color from pale-yellow to wine-red. After boiling for 15 min, the colloids were stirred for another 15 min and cooled to room temperature. Then, the synthesized AuNPs was repeatedly washed with Milli-Q water for three times, and stored at 4 °C unless use. After polishing the working electrode carefully with 1.0, 3.0 and 0.05 μm alumina powder on fine abrasive paper and washed with Milli-Q water, 10.0 μL of AuNPs solution was added onto the working electrode and then dried. The AuNPs modified platform was then washed with PBS washing buffer and characterized by SEM.

2.5. Preparation of the Lu-AuNPs labeled signal $McAb_2$

Lu-AuNPs were prepared by reducing $AuCl_4^-$ ions with luminol according to a reported method [36]. Briefly, after 100 mL of $HAuCl_4$ solution (0.01%, w/w) was heated to boiling point, 1.80 mL of luminol solution (0.01 M) was added rapidly while stirring vigorously. The solution was maintained at boiling point for 30 min, during which time a color change from yellow to purple was observed before a wine-red color was reached. The unreacted reagents were removed via dialysis for 2 days with Milli-Q water for six times under stirring by use of a 3500 molecular weight